Declaration

I, Meng Li, a Group Leader at the Institute for Stem Cell Research, whose address is Institute for Stem Cell Research, The King's Buildings, West Mains Road, Edinburgh EH9 3JQ, United Kingdom, do hereby declare as follows:

I am employed as a Senior Research Scientist at the University of Edinburgh, in the Institute for Stem Cell Research. I am named as an inventor on US Patent Application No. 09/686 880 entitled "Lineage Specific Cells and Progenitor Cells". My qualifications and experience are outlined in the attached C.V.

I am making these statements from my own knowledge in support of the above mentioned US Patent Application.

The Examples provided in the specification of said US Patent Application describe a way of carrying out the invention in which expression of a selectable marker gene was operatively linked to expression of the Sox 2 gene. Using the same techniques as described in the patent specification, I have also assisted the experimental design of a method to make use of the invention by linking expression of a selectable marker to expression of the Sox 1 gene.

Specifically, the open reading frame of the Sox 1 gene was replaced with the coding sequence for enhanced green fluorescent protein (GFP) by homologous recombination. Sox 1 was chosen because it is expressed in neuroepithelial cells along the entire neuraxis but is found in no other tissue in the early to mid-gestation embryo apart from the lens. This highly restricted expression was recapitulated by the GFP reporter in embryos generated from the 46C clone of targeted ES cells. The fidelity of the Sox 1-GFP reporter was maintained *in vitro*. GFP was not detectable in populations of undifferentiated ES cells but, like Sox 1 mRNA and protein became evident in a significant proportion of cells after induction of neural differentiation.

When 46C cells were plated directly on gelatin-coated plastic in a conventional serum-free culture medium with N2 and B27 supplements, more than 70% of cells expressed GFP after 4 days. Furthermore, differentiation into neurons and glia was accompanied by down-regulation of Sox 1. Therefore the number of GFP positive cells at any single time point is likely to be an underestimate of the total incidence of neural determination.

Visualization of GFP combined with immunostaining for the neuroepithelial marker nestin and neuronal marker type III tubulin, confirmed the status of cultures after 9 days as a mixture of neural progenitor cells and differentiated neurons.

FACS purification was used to examine whether GFP expressing 46C cells are distinct from ES cells and constitute lineage-restricted neural progenitor cells. Sorted cells were plated in neural differentiation medium or ES cell culture medium containing LIF. In differentiation media, neurons and glia were obtained at high efficiency from GFP positive cells, but only rarely from GFP negative populations. Cells with overt neuronal morphology immunoreactive for the pan-neuronal marker type III tubulin were apparent

within 2-3 days of plating Sox 1-GFP cells while astrocytes and oligodendrocytes developed over 5-10 days. Conversely, in the presence of serum and LIF, ES cell colonies were readily generated from GFP negative cells but arose only rarely (<0.01%) if at all from the GFP positive population.

Hence, using the same techniques as the invention, Sox 1 was used successfully to select for neural cells.

The marker used in the above Sox 1 work was GFP and neural progenitor cells were selected by FACS purification. However, a number of other marker/selection systems were well-known by April 1998 and would be equally suitable for generating a culture that is purified or enriched in neural progenitor cells. Such marker/selection systems, include use of a β geo marker gene and selection with G418, as described in the patent application in relation to a Sox 2-linked marker.

From my knowledge of the expression profile of the Sox 3 gene in April 1998, I believe that the invention can also be carried out by linking expression of the selectable marker to expression of the Sox 3 gene, resulting also in selection for neural progenitor cells.

From literature reports of other neural specific genes available before April 1998, I also believe the invention can be carried out using the techniques as described in the application as filed using any of those neural specific genes, namely Pax 3, Mash-1, Math-4a, Pax 6, GFAP, Ptx3 (Pitx3), and islet-1/2.

I declare under penalty of perjury that the foregoing is true and correct.

Executed on this 9 day of June 2003 in Edinburgh, United Kingdom

Meng Li

CURRICULUM VITAE

Meng Li, PhD

Institute for Stem Cell Research
University of Edinburgh
King's Buildings
Edinburgh EH9 3JQ
UK

Tel: 44 131 650 5890
Fax: 44 131 650 7773
E-mail: Meng.Li@ed.ac.uk

Education:

May 1992-July 1996 Ph.D.
Institute for Stem Cell Research, University of Edinburgh
PhD thesis studied the function of LIF receptor gene in mouse development.
Supervisor: Dr. Austin Smith

Sep. 1986 - July 1988 M.Sc.
Department of Immunology, Beijing Medical University, Beijing, China.
M.Sc project focused on the analysis of T lymphocyte subpopulations in the thymus and their ability to secret and respond to cytokines.
Supervisor: Prof. Wei-feng Chen

Sept 1980 - July 1986 B.Med.
Beijing Medical University, P.R. China

Present Appointment:

Group leader and MRC Career Development Fellow
Institute for Stem Cell Research, University of Edinburgh
April 2000-present

Previous Appointments:

Postdoctoral Research Fellow
Institute for Stem Cell Research, University of Edinburgh
July 1996-March 2000

Research Assistant
Institute for Stem Cell Research, University of Edinburgh
February 1992-June 1996

Visiting scholar
Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
January 1990-January 1992

Research and Teaching Assistant
Department of Immunology, Beijing Medical University
Beijing, P. R. China
Sept. 1988-Dec. 1990

Teaching experiences:

Honor's elective 'Molecular and cellular approaches to mammalian development'
2000-present.

Grant awards:

04, 2000- 03, 2004. Meng Li, G120/521. MRC Career Development Award on 'Generation, propagation and transplantation of neural precursors derived from embryonic stem cells'. £354,886

04,2000-09,2003. Austin Smith, Meng Li S1763. Glaxo-Wellcome Studentship. 'Molecular characterisation of embryonic stem cell derived neurons'. £20,100.

01,2003-12,2005. Austin Smith, Meng Li, Steve Dunnet. G0100724. MRC Co-operative group component grant. 'Generation and purification of mesencephalic dopamine neurons from embryonic stem cells'. £229,928.

10,2002-09-2005. Meng Li, Timothy Allsopp. G78/7591. MRC Collaborative studentship. £33,990.

Publications:

LAUER, P., METZNER, H.J., ZETTLMEISSL, G., **LI, M.**, SMITH, A.G., LATHE, R. and DICKNEITE, G. (2002). Targeted inactivation of the mouse locus encoding coagulation factor XIII-A: hemostatic abnormalities in mutant mice and characterization of the coagulation deficit. Thromb. Haemost. 88: 967-974

Zhao S, Vougioukalou M, Nichols J, Smith A and **Li M**. Class B transcription factors confer neuroecdodermal fate on Pluripotent Stem Cells. (under review by JCB).

Ying Q, Stavridis M, Griffiths D, **Li M** and Smith A (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture
Nature Biotech (dci:10. 1038/nbt780).

Li M. (2002) Lineage selection for generation and amplification of neural precursor cells.

Methods Mol Biol *185*, 205-215.

Li, M., Price, D., and Smith, A. (2001). Lineage selection and isolation of neural precursors from embryonic stem cells. Symp Soc Exp Biol *53*, 29-42.

Aubert J, Dessolin S, Belmone N, **Li M,** McKenzie FR, Staccini L, Villageois P, Barhanin B, Vernallis A, Smith A, Ailhaud G and Dani C. (1999) Leukemia inhibitory factor and its receptor promote adipocyte differentiation via the mitogen-activated protein kinase cascade. *J Bio Chem* 274:24965-24972.

Dani, Chambers I, Johnstone S, Robertson M, Ebrahimi B, Saito M, Taga T, **Li M,** Burdon T, Nichols J and Smith A.(1998) Paracrine induction of stem cell renewal by LIF-deficient cells: a new ES cell regulatory pathway. *Dev Biol* 203:149-162.

Li M, Pevny L, Lovell-Badge R and Smith A. (1998) Generation of purified neural precursors from embryonic stem cells by lineage selection. *Current Biology*. 8:971-974.

Livesey FJ, O'Brien JA, **Li M,** Smith A, Murphy LJ and Hunt SP. (1997) A Schwann cell mitogen accompanying regeneration of motor neurons. *Nature*. 390:614-618.

Chambers I, Cozens A, Broadbent J, Robertson M, Lee M, **Li M** and Smith A. (1997) Structure of he mouse leukaemia inhibitory factor receptor gene: regulated expression of mRNA encoding a soluble receptor isoform from an alternative 5' untranslated region. *Biochem. J.* 328:879-888.

Li M, Sendtner M, and Smith A. (1995) Essential function of LIF receptor in motor neurons. *Nature*, 378, 724-727.

Mountford P, Zevnik B, Duwel A, Nichols J, **Li M,** Dani C, Robertson M, Chambers I, and Smith A. (1994). Dicistronic targeting constructs: reporters and modifiers of mammalian gene expression. *Proc. Natl. Acad. Sci. USA*, 91, 4303-4307.

Li M and Bernard O. (1992). FDC-P1 myeloid cells engineered to express firobroblast growth factor receptor 1 proliferate and differentiate in the presence of fibroblast growth factor and heparin. *Proc. Natl. Acad. Sci. USA*, 89, 3315-3319.

Metcalf D, Nicola N, Gough NM, Elliott M, McArthur G and **Li M.** (1992) Synergistic suppression: Anomalous inhibition of the proliferation of factor-dependent hemopoietic cells by combination of two colony-stimulating factors. *Proc. Natl. Acad. Sci. USA*,89:2819-2823.

Bernard O, **Li M,** and Reid H. (1991). Expression of two different forms of fibroblast growth factor receptor-1 in different mouse tissues and cell lines. *Proc. Natl. Acad. Sci. USA*, 88, 7625-7629.

Patents:

Dickneite, G., Metzner, H., Zettlmeissl, G., Grundmann, U., Lathe, R., Smith, A. G. and **Li, M** (1998)
A transgenic coagulation factor XIII defective animal and its use for testing wound healing and bleeding

Patent application no. 98117978.1-2106

Smith, A. G. and **Li,M**. (1999)
Lineage specific cells and progenitor cells
PCT/GB99/01136

Recent and forthcoming conference presentations:

Poster presentation
Forum of European Neuroscience (FENS) 2000, 24-28th June, 2000.
Brighton, UK.

Speaker
SEB Annual Symposium, Brain Stem Cells: Development and Regeneration.
1-3 Aug, 2000, Cambridge, UK.

Speaker
The 12th Network of European CNS Transplantation and Restoration, 30/11-2/12, 2001.
Brussels, Belgium.

Speaker
The 21st Joint Meeting of the British Endocrine Societies, 8-11 April 2002. Harrogate, UK.

Speaker
SPRING scientific conference, 11 March, 2003. London.